Supporting Material

NMR-based metabolomic for frauds detection and quality control of oregano samples

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Position	Number of integrated protons	^{1}H, δ, m, J (Hz)	^{13}C HSQC	HMBC correlations	COSY correlations
1A	C	-	124.70	-	-
2A	C	-	124.77	-	-
3A	C	-	142.11	-	-
4A	C	-	146.66	-	-
5A	CH	6.81, d, *J* = 8.54	117.05	4A; 6A	6A
6A	CH	6.94, d, *J* = 8.54	122.43	1A; 3A; 5A; 7A	5A
7A	CH	6.91, d, *J* = 16.27	142.59	1A; 6A; 8A; 9A	8A
8A	CH	5.79, d, *J* = 16.27	115.52	7A	7A
9A	C	-	168.05	-	-
1B	C	-	129.90	-	-
2B	CH	6.17, d, *J* = 2.33	116.74	2B; 3B; 7Bα; 7Bβ	6B
3B	C	-	142.10	-	-
4B	C	-	143.51	-	-
5B	CH	6.35, d, *J* = 8.54	115.94	1B; 4B	6B
6B	CH	6.00, d *J1* = 8.54; *J2* = 2.33	120.27	2B; 3B	5B; 2B
7Bα	CH	2.92, dd, *J1* = 14.70; *J2* = 12.11	36.41	1B; 6B	7Bβ
7Bβ	CH	2.49, dd, *J1* = 14.70; *J2* = 3.94	36.41	1B; 2B; 6B; 8B	7Bα; 8B
8B	CH	4.83, dd, *J1* = 12.11; *J2* = 3.94	77.99	-	8Bβ
1C	C	-	123.16	-	-
2C	CH	6.84, d, *J* = 2.33	113.03	4C; 6C; 7C	
3C	C	-	146.92	-	-
4C	C	-	143.80	-	-
5C	CH	6.82, d, *J* = 8.54	116.93	1C; 3C	6C
6C	CH	6.74, dd, *J1* = 8.54; *J2* = 2.33	117.65	2C; 4C; 7C	5C
7C	CH	5.90, d, *J* = 5.82	56.74	3C; 8C; 9C	8C
8C	CH	4.33, d, *J* = 5.82	86.90	2A; 9C	7C
9C	C	-	172.47	-	-
1D	C	-	130.47	-	-
2D	CH	6.85, d, *J* = 2.33	116.77	4D; 6D	-
3D	C	-	143.94	-	-
4D	C	-	142.93	-	-
5D	CH	6.86, d *J* = 8.54	166.57	1D: 3D	6D
6D	CH	6.70, dd, *J1* = 8.54; *J2* = 2.33	122.21	2D; 4D	5D
7Dα	CH	3.04, dd, *J1* = 14.44; *J2* = 3.54	36.75	1D	7Dβ

7Dβ	CH	2.85, dd, *J1* = 14.44; *J2* = 10.10	36.75	1D; 2D; 6D; 8D	7Dα; 8D
8D	CH	4.93, dd, *J1* = 10.10; *J2* = 3.54	76.64	1D	7Dβ

Table S1. Summary of NMR experiments performed in order to elucidate the structure of salvianolic acid B. Sample was solubilized in CD_3OD. Table reports: number of integrated protons, chemical shift (δ), splitting pattern, coupling constants (in Hz), HSQC, HMBC and COSY correlations.

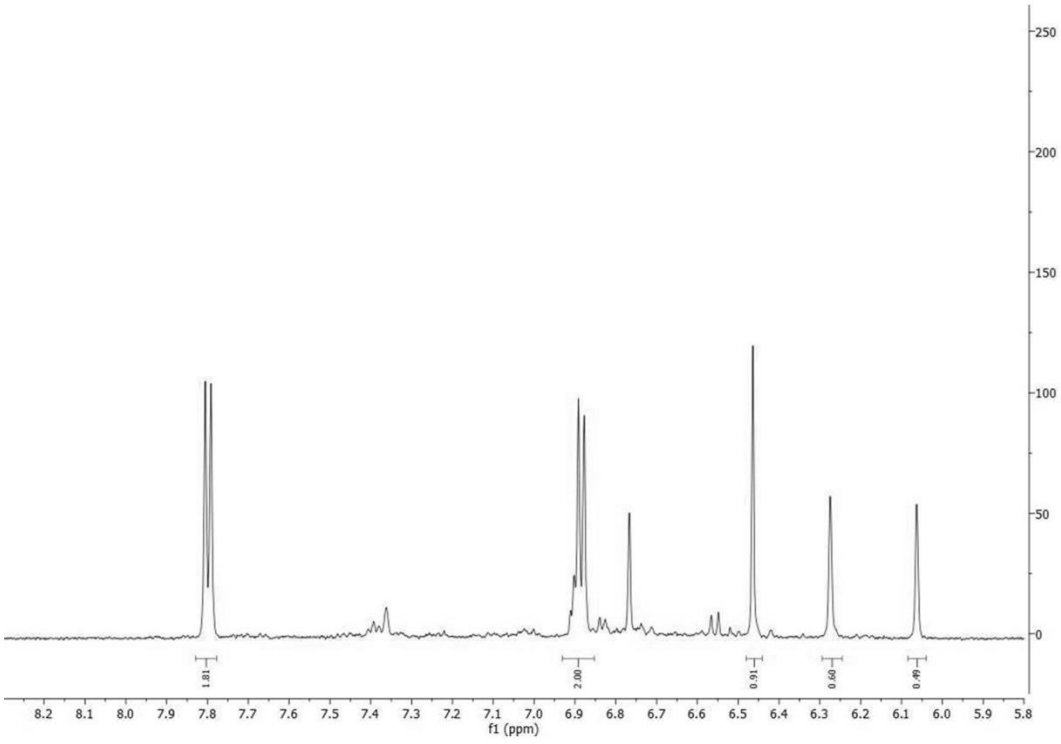


Fig. S1 ^1H NMR spectrum obtained for apigenin (in fraction 114) solubilized in CD_3OD.

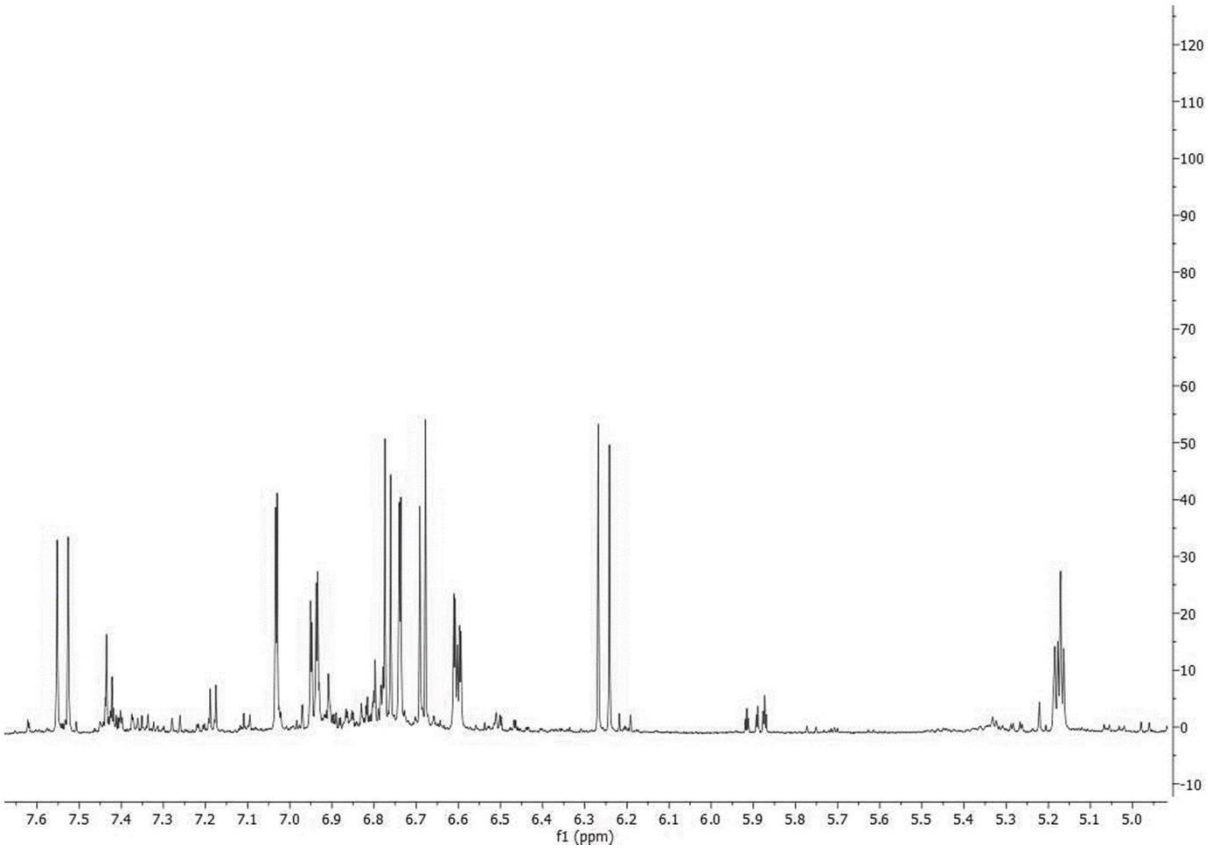


Fig. S2. ^{1}H NMR spectrum obtained for rosmarinic acid (found in EtOAc fraction from *Oregano vulgare*) solubilized in CD_3OD.

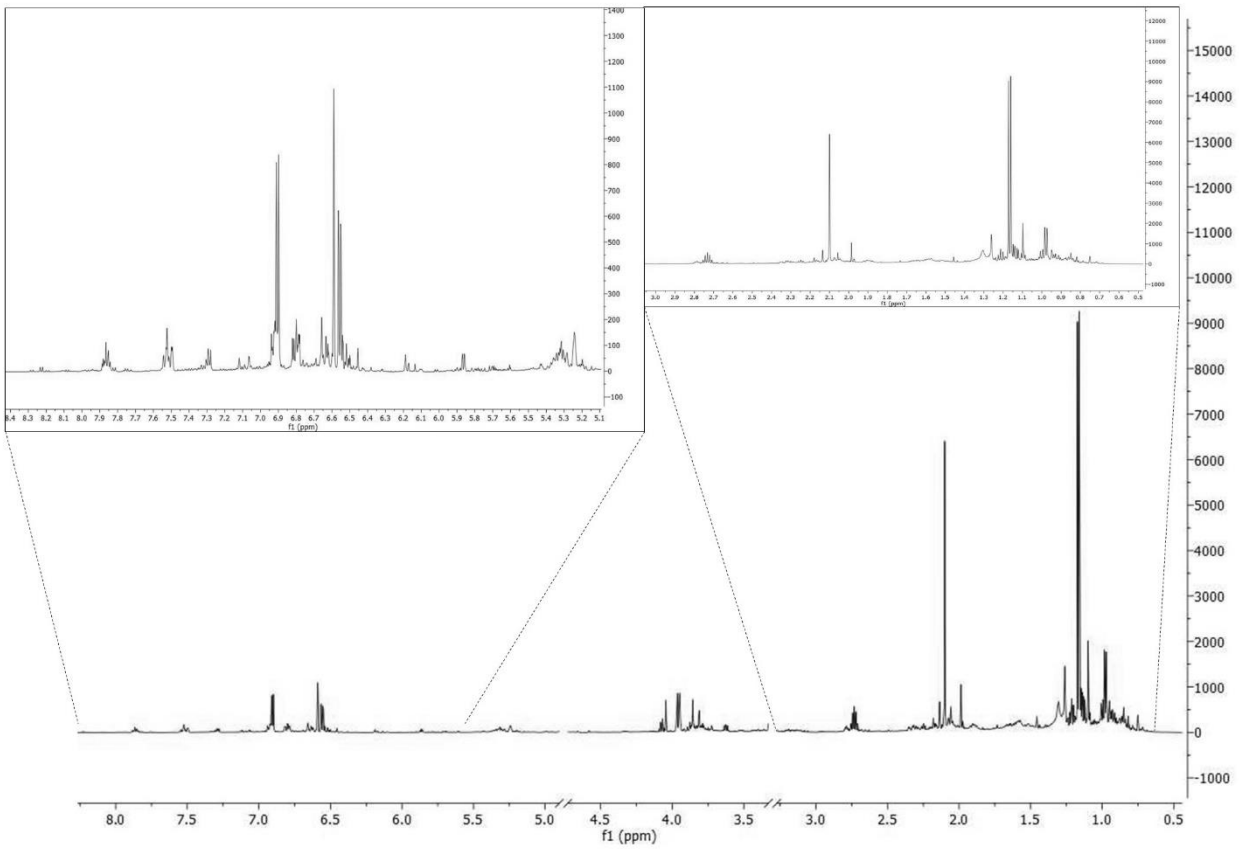


Fig. S3 ^{1}H NMR spectrum obtained for thymol, and p-cymene (found in $CHCl_3$ fraction from *Oregano onites*) solubilized in CD_3OD.

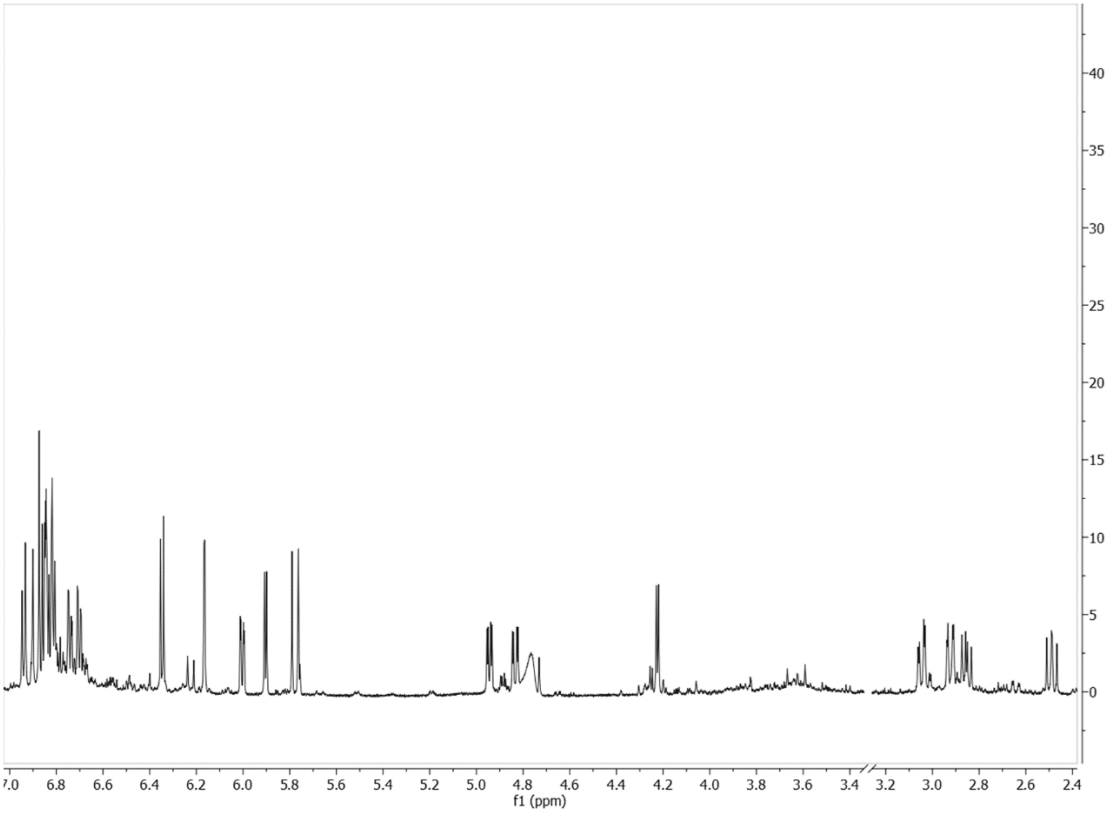


Fig. S4 ^{1}H NMR spectrum of salvianolic acid B solubilized in CD_3OD.

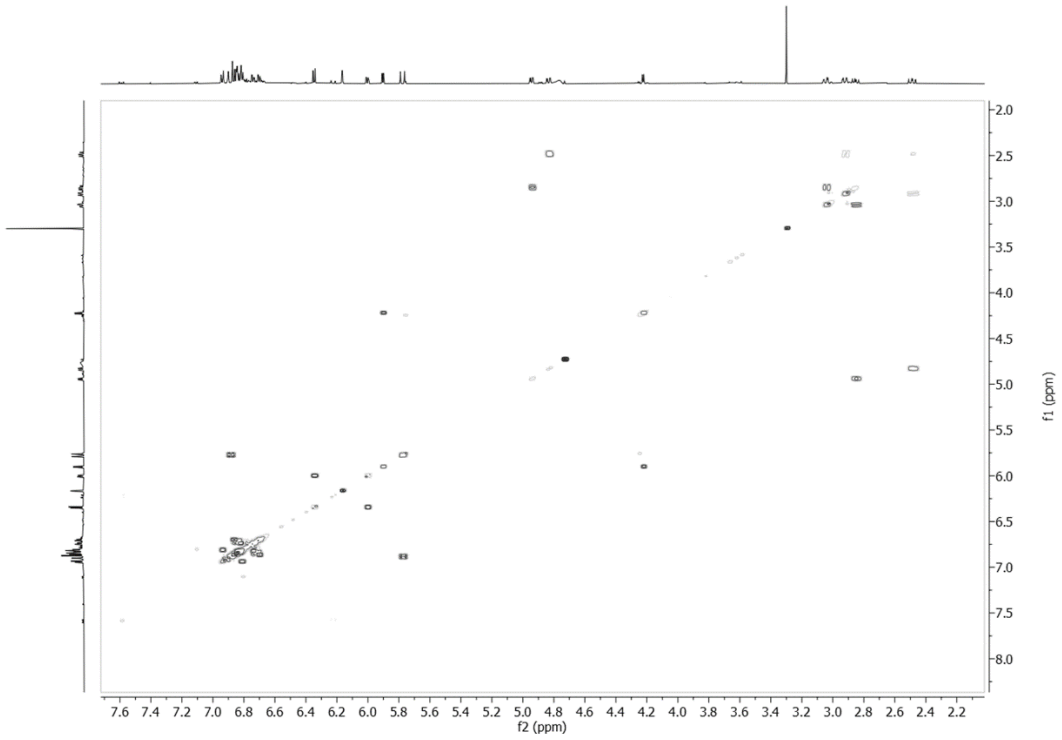


Fig. S5 NMR COSY spectrum of salvianolic acid B solubilized in CD_3OD.

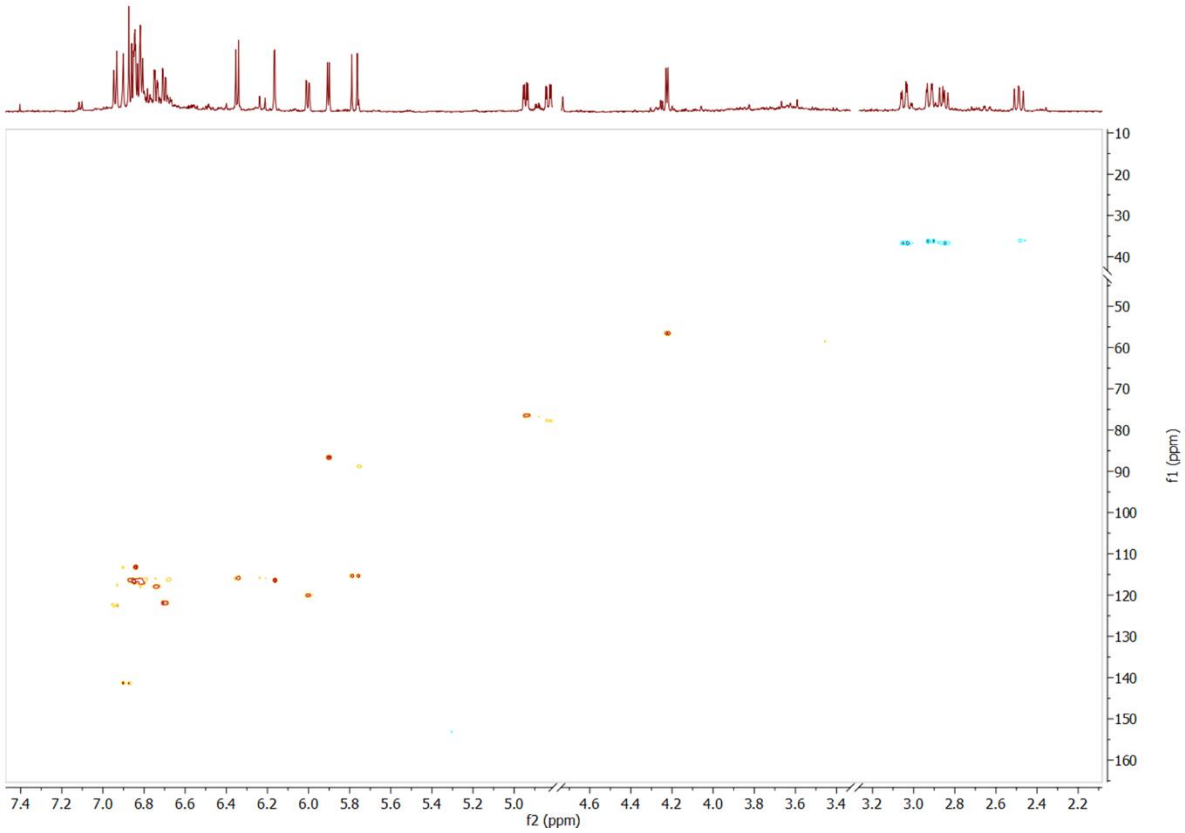
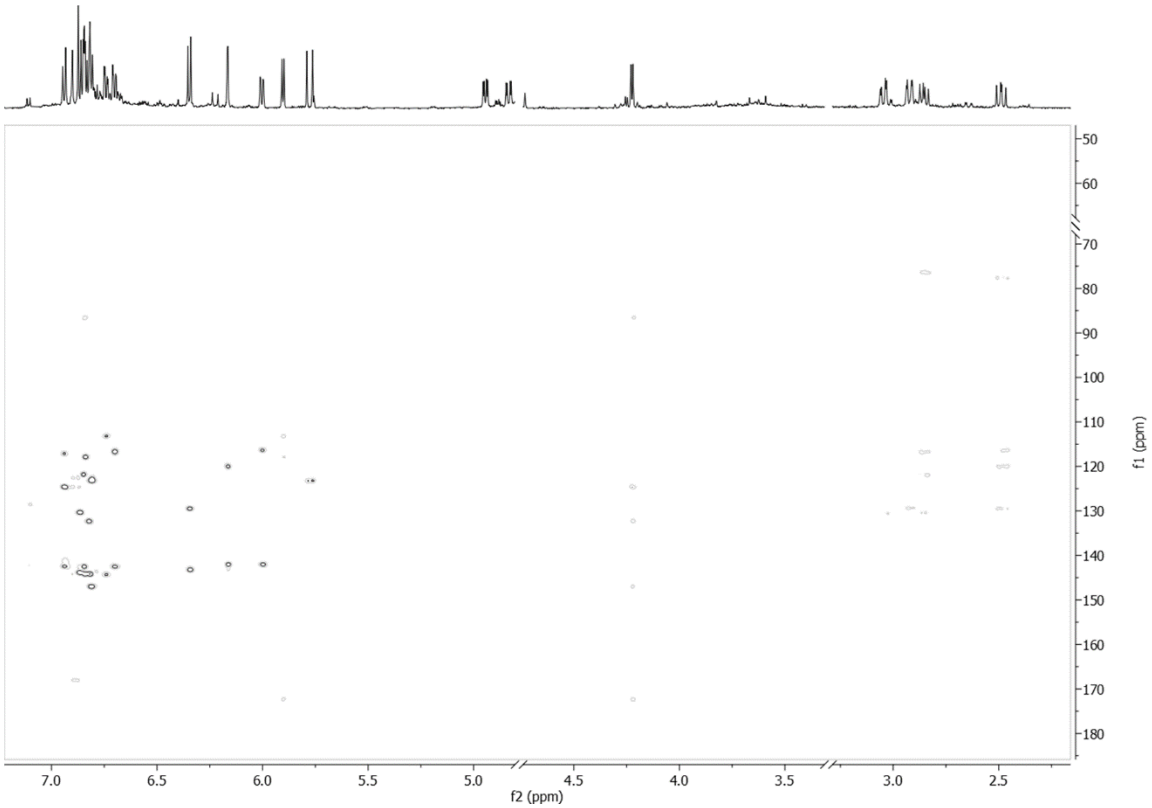


Fig. S6 NMR HSQC spectrum of salvianolic acid B solubilized in CD_3OD.



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79 **Fig. S7** NMR HMBC spectrum of salvianolic acid B solubilized in CD_3OD.

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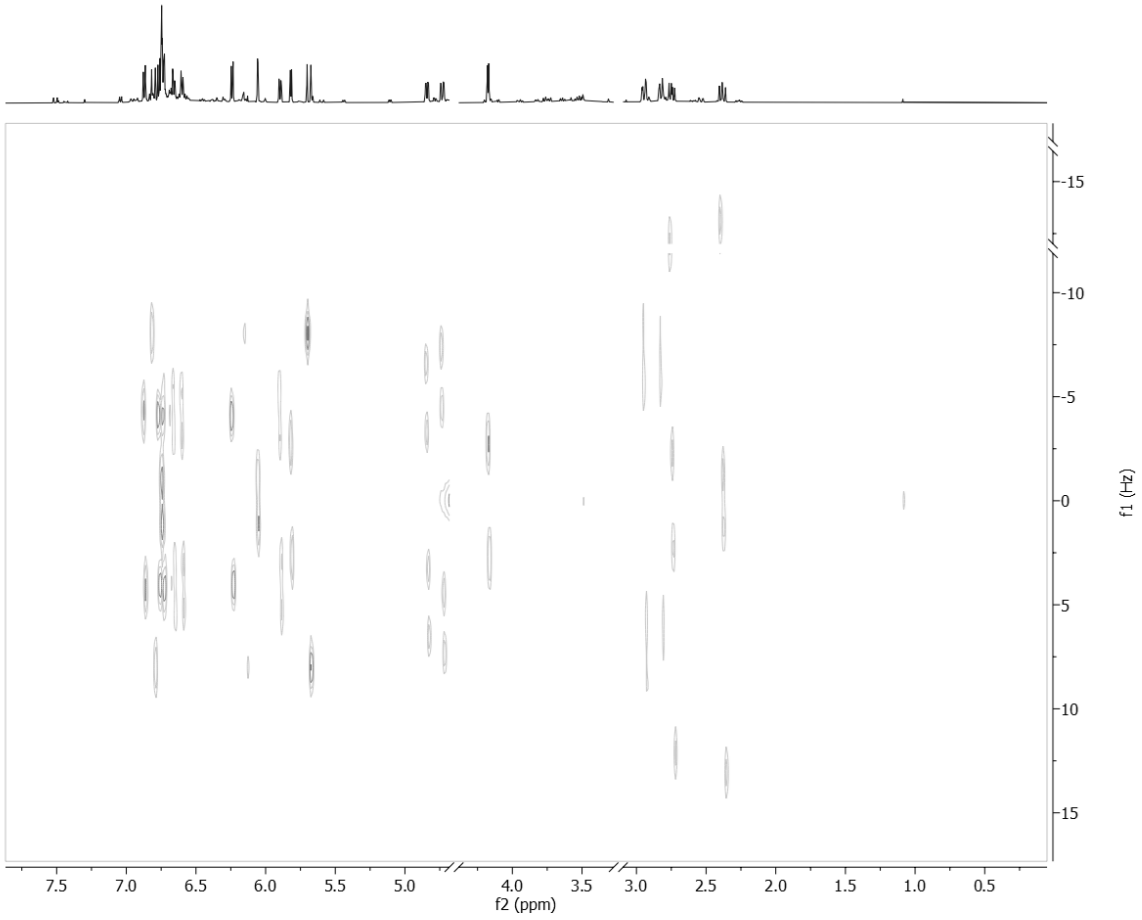


Fig. S8 NMR *J-res* spectrum of salvianolic acid B solubilized in CD_3OD.

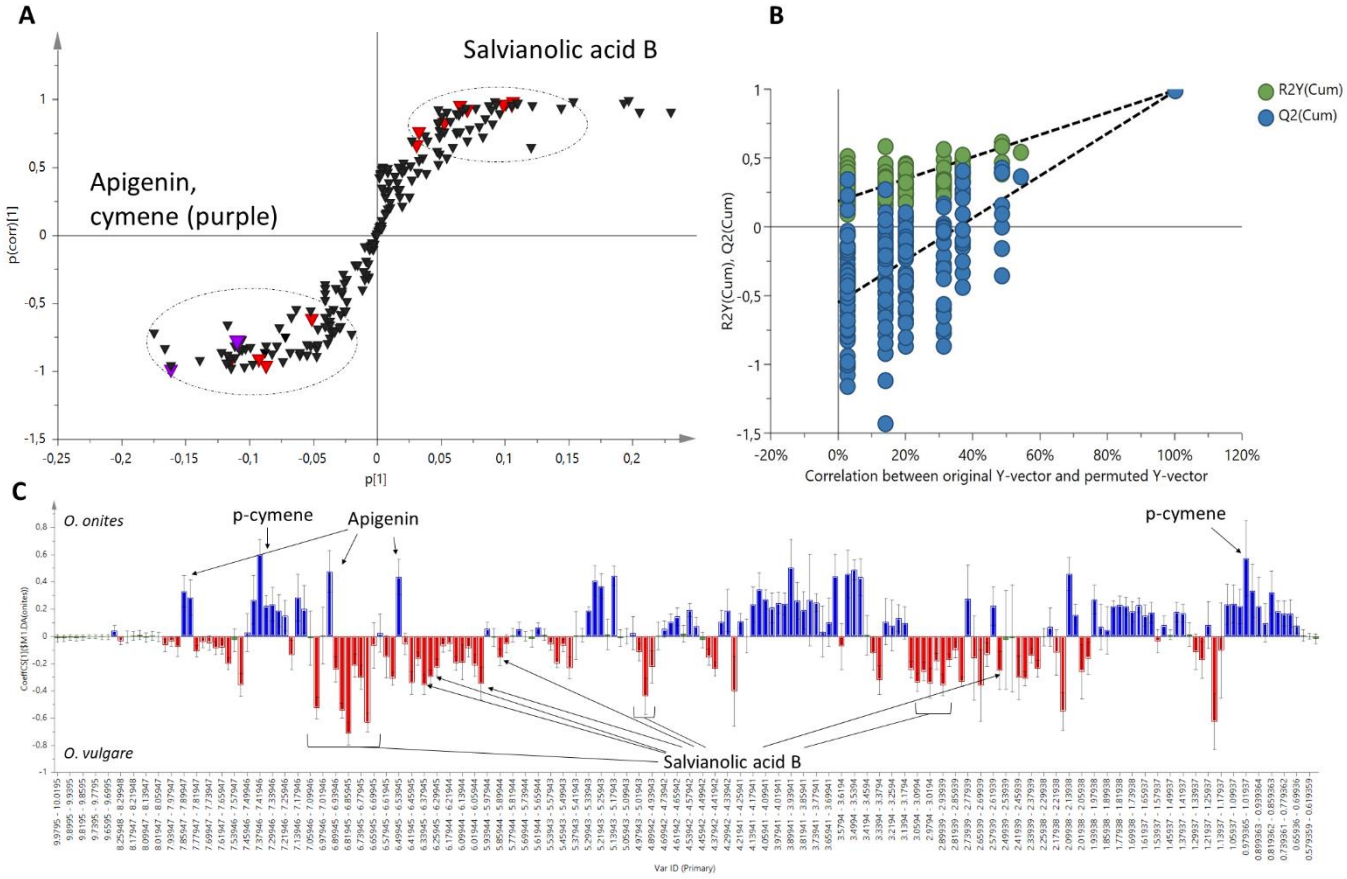
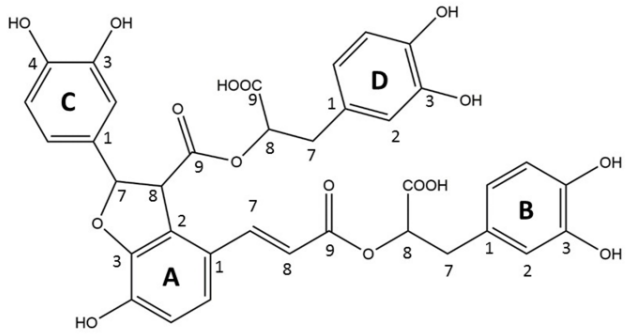
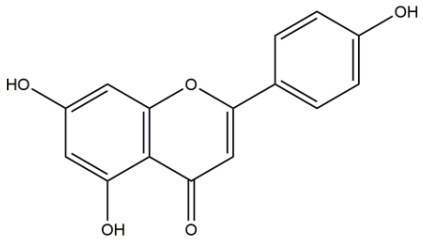


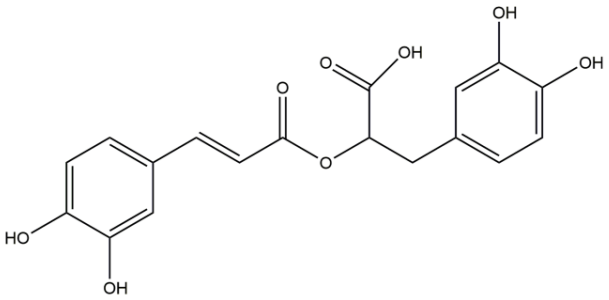
Fig. S9 A) S-plot from OPLS-DA model showing the most important spectral bins for the discrimination between the two commercial species of oregano. **B)** Result of the permutation test of the OPLS-DA model. **C)** Loading column plot from OPLS-DA model.



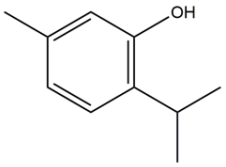
salvianolic acid B



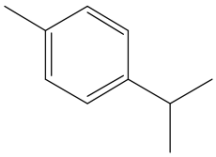
apigenin



rosmarinic acid



thymol



p-cymene

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96 **Fig. S10** Main biomarkers found in this work.

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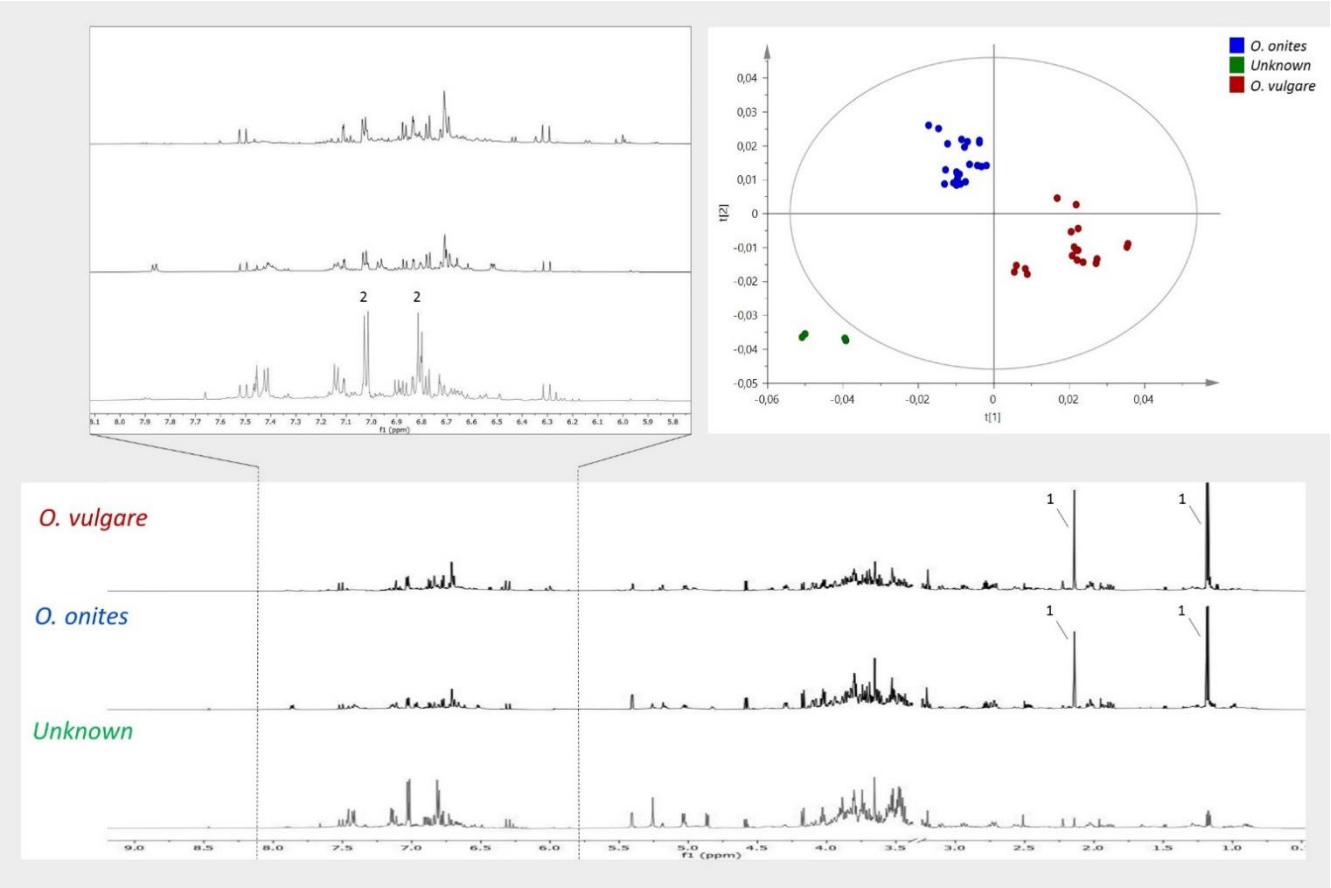

Fig. S11. PCA score scatter plot where unknown species (green dots) are distinguished from marketable oregano and placed as outliers by the model. As showed by the ^{1}H NMR profiles, the lack of thymol (1) and other essential oil components and the high amount of tyrosine (2) were the main differences between these samples and the marketable oregano species.

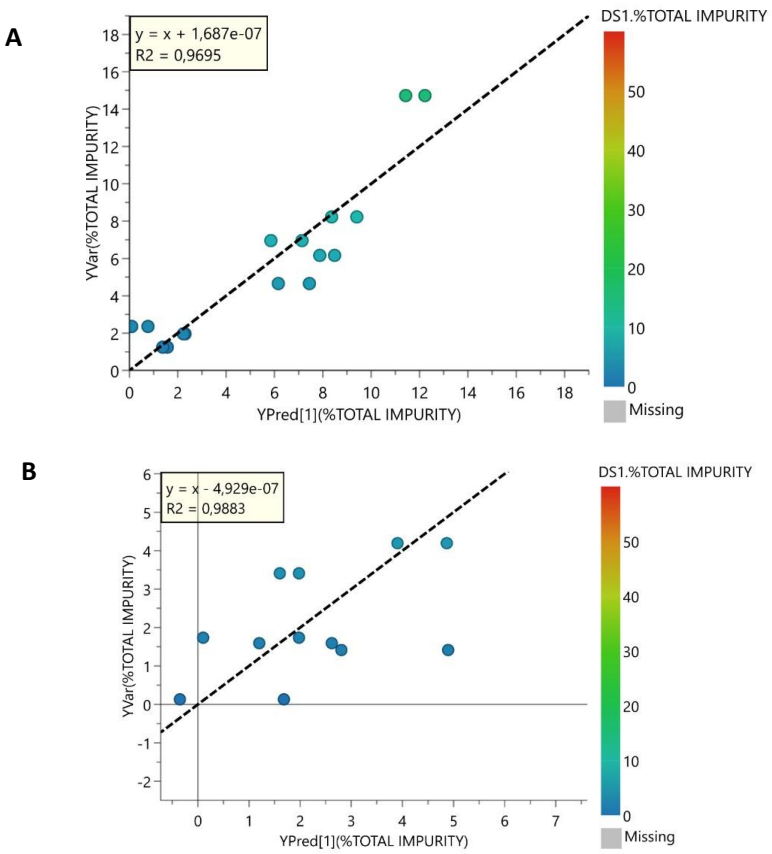

Fig. S12 Extended regions of observed *vs* predicted plots from OPLS models (*y* = % of total impurity) of **A)** *Origanum vulgare* B) *Origanum onites*

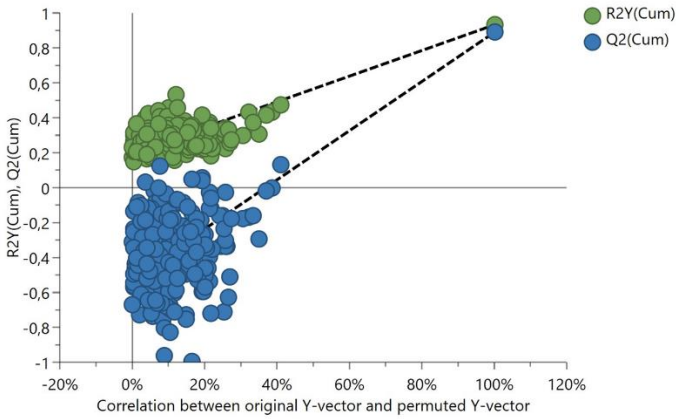

Fig. S13 Graphic obtained by permutation test of the OPLS model built using as *y* variable the % of cistus contamination.

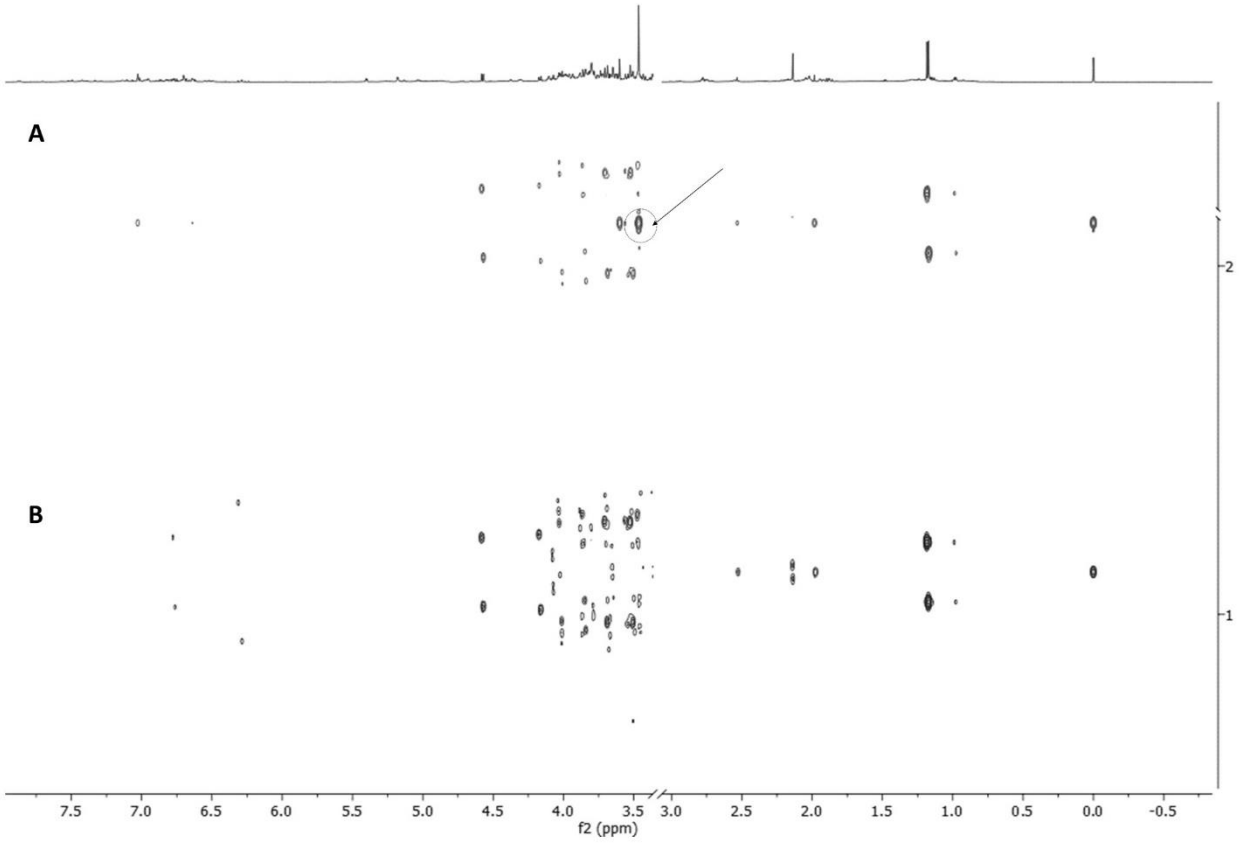

Fig. S14 *J-res* spectra of Oregano contaminated with cistus (A) and pure oregano (B). The contaminated sample presents a singlet at δ 3.57.